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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

This report details the progress made during the first year of ONR support of a research project focused on an examination of the formation and degradation of sulfonates by bacteria. Described are findings that both phototrophic bacteria and non-phototrophic ones can metabolize soluble sulfonates such as cysteate, isethionate, and taurine. In some instances the entire organosulfur compound can be utilized (degraded) by one or another bacterium, in other instances only the sulfonate-sulfur is assimilated, the fate of the remainder of the molecule is unknown. Plans for the second year effort on this project are outlined.

Keywords: biotransformation

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ANNUAL REPORT:

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PRINCIPAL INVESTIGATORS: Edward R. Leadbetter, Walter Godchaux III

CONTRACTOR: The University of Connecticut, Storrs

CONTRACT TITLE: Formation and Fate of Bacterial Sulfonates

START DATE: 1 January 1988

DATE OF REPORT: 5 JANUARY 1989

RESEARCH OBJECTIVES: **a.** to isolate and characterize bacteria able (**i**) to utilize sulfonate-sulfur for biosynthesis or (**ii**) to carry out dissimilative sulfonate reduction, then to assess the physiology and biochemistry of these two different processes. **b.** to examine the routes of sulfonate-S formation in the simple gliding bacteria that are known to biosynthesize the sulfonate, cysteate, in significant quantities.

PROGRESS:

a. Examination of the biotransformation (biodegradation) of sulfonates has been focused on two physiological types of bacteria--those phototrophic bacteria able to live "facultatively" either by phototrophy in anaerobic environments or by light-independent respiration under aerobic conditions, as well as non-phototrophic (chemoorganotrophic) bacteria that are strictly respiratory; in this latter category anaerobic respiration with nitrate has also been examined. Thus far the bulk of the bacteria isolated have been from fresh-water and soil habitats.

PHOTOTROPHS. Rhodobacters and rhodopseudomonads (Rhodospirillaceae) have been obtained by enrichment culturing [in the light, anaerobically] using sulfonates (e.g., cysteate, isethionate, taurine) as sole sources of S, and the ability of the bacteria to utilize sulfonate-S for assimilative purposes has been compared under the two growth conditions--phototrophic and respiratory. Several salient facts emerge: sulfonate-S and sulfate-S support equal amounts of growth [thiosulfate did not serve effectively]; a nutritional specificity, or selectiveness, exists in some strains (i.e., not all three of the sulfonates can serve as S sources), while other strains are nutritionally versatile; under phototrophic conditions many, but not all, strains are able to utilize the entire sulfonate molecule as sole source of C, S, and N. This is the first demonstration of the biotransformation of sulfonates under phototrophic, anaerobic conditions!

Three cyanobacteria--a strain each of Synechococcus, Anabena, and Nostoc--have been examined for their ability to utilize the sulfonate taurine as sole source of S for their oxygenic phototrophic growth; only Anabena and Nostoc were able to do so, and the latter could do so when grown either phototrophically or heterotrophically.

CHEMORGANOTROPHS. Pseudomonads and other aerobic bacteria. The enrichment culture and isolation of chemoorganotrophs able to utilize sulfonates as sole source of C, S, E (energy) and (where applicable) N has led to the demonstration that the isolates are able to attack and utilize taurine as sole C, N, S, and energy source, and that the majority of the strains also are able to so utilize isethionate [excepting N utilization] ; strangely, only one isolate is able to degrade cysteate.

b. Cysteate synthesis and its regulation.

Although on comparative biochemical grounds one would expect the biosynthesis of cysteate to proceed from the amino acid cysteine, via an oxidation of the sulfhydryl group to a sulfinate, thence to the sulfonate moiety, our earlier studies demonstrating that the carbon of cysteine did not appear in cysteate indicated that this expected route was not followed in Cytophaga johnsonae. Isolation of a mutant unable to synthesize cysteine, but still able to form sulfonolipids of which cysteate is the "head-group" precursor, has led to a quite clear demonstration that the biosynthesis of cysteate proceeds by a pathway other than one involving cyteine's carbons. A number of possible precursors are being evaluated experimentally.

As one means of establishing the pathway for cysteate synthesis, a kinetic analysis of sulfate uptake and its incorporation into low molecular weight intracellular metabolites was undertaken. Using wild-type C. johnsonae, we have demonstrated that cysteate is indeed a major S-containing metabolite in these bacteria and, in addition that these organisms possess regulatory features for sulfate uptake and assimilation that seem quite unlike those studied in enteric, and other, bacteria and in plants and thus may possess a novel pattern of regulation of sulfur metabolism.

WORK PLAN (YEAR 2).

a. PHOTOTROPHS and AEROBIC CHEMOORGANOTROPHS. The identification of the bacteria already isolated should be completed, as should also the isolation and characterization of marine isolates. Thus for both bacterial groups then, it may be possible to begin focusing on the nature of the enzyme(s) involved in cleaving the C-S bond of sulfonates, and determining whether the enzymes involved are identical in those instances where the S is used only for assimilative purposes as contrasted with the cases where the entire sulfonate molecule is degraded.

SULFONATE RESPIRATION. Although stable enrichment cultures that seemingly effect the reduction of sulfonates (the only known electron acceptor added) are in hand, we have had no luck yet in obtaining pure cultures that carry out this transformation(s). Efforts to do so will continue, using more stringent anaerobic techniques than have heretofore been employed by us.

b. CYSTEATE BIOSYNTHESIS. During year two we expect to have obtained definitive evidence supporting, or ruling out, the involvement of several prospective precursor metabolites of cysteate; aspartate is the molecule that, on the basis of present experimental evidence, seems the most likely candidate. Once evidence from study of intact cells is indicative of the likely biosynthetic pathway we will turn to examination of cell-extracts in order to provide firm evidence of the route for biosynthesis.

INVENTIONS. none

PUBLICATIONS, REPORTS.

1. Cysteine is not an obligatory intermediate in the biosynthesis of cysteate by Cytophaga johnsonae. D. F. Gilmore, W. Godchaux III, E. R. Leadbetter, Biochem. Biophys. Res. Commun., SUBMITTED

2. Regulation of cysteate synthesis in the gliding bacterium Cytophaga johnsonae. D. F. Gilmore, W. Godchaux, E. R. Leadbetter. SUBMITTED for presentation at the Annual Meeting, Amer. Soc. Microbiol., 1989.

3. Bacterial utilization of sulfonates. C. M. Green, A. P. Seitz, E. R. Leadbetter. SUBMITTED for presentation, Annual Meeting, Amer. Soc. Microbiol., 1989.

4. A progress report covering six months' research was prepared and mailed, in June 1988, to those on the distribution list.

TRAINING ACTIVITIES. Graduate students Catherine Greene, David Gilmore, Thomas Pitta, Lisa Gorski and Maria Uria have been supported in whole or part by this contract. An undergraduate student, Angelica Seitz, has worked on this project, and will continue to do so during at least the first six months of year two, on a part time basis.

Demographic data requested: Women, three graduate, 1 undergraduate student; Non-citizens: 1 (M. Uria, native of Bolivia).

AWARDS/FELLOWSHIPS:

ERL was an Inter-Academy Exchange Fellow of the U. S. and Hungarian National Academies of Science for three months in Fall 1988; site was at the Biological Research Center, Szeged, Hungary, where cyanobacterial stress responses and sulfonate utilization were studied.

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